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**Title**

Rhizosphere activity and atmospheric methane concentrations drive variations of methane fluxes in a temperate forest soil

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## Abstract

Aerated soils represent an important sink for atmospheric methane ( $\text{CH}_4$ ), due to the effect of methanotrophic bacteria, thus mitigating current atmospheric  $\text{CH}_4$  increases. Whilst rates of  $\text{CH}_4$  oxidation have been linked to types of vegetation cover, there has been no systematic investigation of the interaction between plants and soil in relation to the strength of the soil  $\text{CH}_4$  sink. We used quasi-continuous automated chamber measurements of soil  $\text{CH}_4$  and  $\text{CO}_2$  flux from soil collar treatments that selectively include root and ectomycorrhizal (ECM) mycelium to investigate the role of rhizosphere activity as well as the effects of other environmental drivers on  $\text{CH}_4$  uptake in a temperate coniferous forest soil. We also assessed the potential impact of measurement bias from sporadic chamber measurements in altering estimates of soil  $\text{CO}_2$  efflux and  $\text{CH}_4$  uptake. Results show a clear effect of the presence of live roots and ECM mycelium on soil  $\text{CO}_2$  efflux and  $\text{CH}_4$  uptake. The presence of ECM hyphae alone (without plant roots) showed intermediate fluxes of both  $\text{CO}_2$  and  $\text{CH}_4$  relative to soils that either contained roots and ECM mycelium, or soil lacking root- and ECM mycelium. Regression analysis confirmed a significant influence of soil moisture as well as temperature on flux dynamics of both  $\text{CH}_4$  and  $\text{CO}_2$  flux. We further found a surprising increase in soil  $\text{CH}_4$  uptake during the night, and discuss diurnal fluctuations in atmospheric  $\text{CH}_4$  (with higher concentrations during stable atmospheric conditions at night) as a potential driver of  $\text{CH}_4$  oxidation rates. Using the high temporal resolution of our data set, we show that low-frequency sampling results in systematic bias of up-scaled flux estimates, resulting in under-estimates of up to 20% at our study site, due to fluctuations in flux dynamics on diurnal as well as longer time scales.

## Introduction

Biogenic trace gases such as carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) play a pivotal role in global climate change (Ciais et al., 2013; Tian et al., 2016). Anthropogenically driven increases in atmospheric CO<sub>2</sub> from fossil fuel combustion and land-use change are the main drivers of climate change. Increasing atmospheric CH<sub>4</sub> concentrations are now thought to contribute 20% of the total greenhouse gas warming (Ciais et al., 2013; Myhre et al., 2013). For anthropogenic CH<sub>4</sub> emission sources, rice cultivation, ruminants, landfills, and gas evasion during fossil fuel extraction dominate (Ciais et al., 2013; Myhre et al., 2013). Methane oxidation in upland soils represent an important sink for atmospheric CH<sub>4</sub>, but poor constraints on the uptake of atmospheric CH<sub>4</sub> by soil microorganisms contributes to overall uncertainty in the global atmospheric CH<sub>4</sub> budget, and predictions of how soil-atmosphere feedbacks may modulate future changes in atmospheric CH<sub>4</sub> concentrations (Kirschke et al., 2013; Nisbet et al., 2014). Similarly, whilst the dynamics and drivers of CO<sub>2</sub> exchange from terrestrial ecosystems are reasonably well understood (Jung et al., 2011), there remain significant uncertainties around feedbacks between plants, soil microbes, and the potential role of rhizosphere priming effects (Talbot et al., 2013).

Trace gas fluxes between soil and atmosphere are directly influenced by the spatial and temporal variations in biotic and abiotic conditions and biogeochemistry. For CO<sub>2</sub> in particular, the role of temperature and soil water availability on heterotrophic decomposition of soil organic matter is well described (Barron-Gafford et al., 2011; Moyano et al., 2012), and also the role of autotrophic (root derived) substrate supply to the rhizosphere is accepted as an important driver of soil metabolic activity (Högberg et al., 2001; Singh et al., 2004). There is further an increasing acceptance of the significance of ectomycorrhizal (ECM) hyphae as recipients of autotrophic C supply in belowground carbon cycling of temperate forests (Subke et al., 2011; Heinemeyer et al., 2012). Soil C priming, whereby plant-derived substrates enhance heterotrophic SOM decomposition by soil microorganisms, has also been described in a wide range of soil conditions (Kuzyakov et al., 2000; Subke et al., 2004), underlining an important interaction between autotrophic and heterotrophic soil C turnover. For CH<sub>4</sub> dynamics, there is a lack of knowledge regarding the interaction with belowground plant C supply. Whilst the influence of soil conditions such as water content, redox potential and (to a lesser extent) temperature are generally well

described, we lack field-based data for interactions of methane oxidation with autotrophic C supply in upland soils. It is known that low molecular weight compounds (i.e. single carbon, or 'C1' molecules) exuded from roots or ectomycorrhizal hyphae support a diverse bacterial community in the rhizosphere (Fransson et al., 2016), potentially including atmospheric CH<sub>4</sub> oxidizers. This is because methanotrophs are able to subsist on other simple C1 compounds (e.g. methanol, formaldehyde, formate) when CH<sub>4</sub> is scarce (Hanson and Hanson, 1996). As a consequence, the greater diversity and availability of labile C compounds in the rhizosphere may buffer methanotrophic populations during periods when CH<sub>4</sub> availability is low. Moreover, mineralization of nutrients from soil organic matter in the rhizosphere may alleviate nutrient limitation among methanotrophs, promoting larger and more active methanotrophic populations (Bodelier and Laanbroek, 2004; Veraart et al., 2015).

One of the main methodological challenges lies in understanding how trace gas fluxes respond to changes in biotic and abiotic variables that fluctuate over relatively short timescales (e.g. hours to days) (Groffman et al., 2009; Savage et al., 2014). These phenomena are difficult to study because of the limitations imposed by conventional low frequency sampling techniques. For example, transient weather phenomena – such as rainfall events, atmospheric pressure variations, or changes in wind speed – can profoundly alter soil-atmosphere fluxes by affecting gas transport processes (Tokida et al., 2007; Yano et al., 2014; Redeker et al., 2015) or rates of biological activity (Groffman et al., 2009; Liptzin et al., 2011; Heinemeyer et al., 2012; Yano et al., 2014). Diurnal fluctuations in temperature, moisture, irradiance, or atmospheric conditions can also modulate trace gas fluxes through direct or indirect effects on the metabolic activity of plants and microorganisms (Subke and Bahn, 2010; Baldocchi et al., 2012; Hatala et al., 2012; Wang et al., 2013). Sporadic trace gas measurements run the risk of systematic bias of true flux estimates, as fluctuations in drivers are not captured appropriately, and specific times of day when measurements are typically carried out (e.g. around midday) represent only a partial sample of diurnal conditions or flux dynamics. Whilst there are some investigations of impacts of sampling intervals and bias from limited diurnal sampling windows (Savage et al., 2014; Ueyama et al., 2015), a further quantification of uncertainty associated with manual/sporadic vs. automated/continuous measurements is necessary to capture site specific conditions and inform comparisons among studies.

Methane oxidation in well-drained soils, in particular, is significantly affected by CH<sub>4</sub> availability (Bender and Conrad, 1992; Hanson and Hanson, 1996; Tate et al., 2012), which may rapidly fluctuate based on local meteorological conditions (Baldocchi et al., 2012; Redeker et al., 2015). However, evidence for a concentration-based effect on atmospheric CH<sub>4</sub> oxidation has largely been obtained from laboratory incubations using high concentrations of CH<sub>4</sub>, which exceed values normally observed in well-drained, aerobic soils, mimicking instead microaerophilic or near-anaerobic wetland conditions (Bender and Conrad, 1992; Teh et al., 2006; Templeton et al., 2006; Tate et al., 2012; Malghani et al., 2016). Field studies of CH<sub>4</sub> concentration effects under ambient conditions are far less common, because past work on atmospheric CH<sub>4</sub> oxidation has focused on isotope fractionation effects rather than on uptake kinetics (King et al., 1989; Reeburgh et al., 1997). Thus, it is unclear if fluctuations in atmospheric CH<sub>4</sub> concentrations significantly influence CH<sub>4</sub> uptake *in situ* because of the prevalence of other environmental drivers (e.g. moisture, temperature) and the narrow range over which atmospheric CH<sub>4</sub> concentrations typically vary.

Here we present the results from a quasi-continuous automated flux chamber experiment that investigated the effects of rapid, short-term fluctuations (i.e. hourly) in environmental variables and the presence or absence of plant roots and/or extra radical ECM mycelium in modulating soil-atmosphere fluxes of CO<sub>2</sub> and CH<sub>4</sub> from a temperate forest soil. The aim of this research was to: (a) establish if the presence of an intact rhizosphere significantly altered rates of trace gas exchange; (b) determine if rapid, short-term fluctuations in environmental variables influenced CO<sub>2</sub> and CH<sub>4</sub> fluxes in temperate forest soils; and (c) identify potential measurement bias from discontinuous sampling strategies.

## Methods

### *Study site*

The field site is a 19-year-old (in 2009) forest stand dominated by *Pinus contorta* and *Pinus sylvestris* (approximate height: 6 to 8 m) with occasional *Betula pendula* but no ground cover, situated approximately 8 km south of York, UK (53°54'38"N 0°59'54"W). The site has

a well-draining sandy gley podzol overlain by a thin (c. 3 cm on average) organic horizon and a litter layer of between 1 and 2 cm. The pH (H<sub>2</sub>O) of the A<sub>h</sub> horizon is approx. 3.5 (Heinemeyer et al., 2011).

## *Experimental design*

To address the influence of root and rhizosphere C supply to soil, we included three contrasting rhizosphere treatments (n=4 per treatment): 1) a *Soil only* treatment (hereafter referred to as '*S*'); a *Soil plus extramatrical ECM mycelium* treatment (hereafter referred to as '*SM*'); and a *Soil plus roots plus extramatrical ECM mycelium* treatment (hereafter referred to as '*SMR*').

For the *S* treatment, PVC pipe sections (20 cm diameter, 35 cm long) were inserted into the soil to a depth of 30 cm. Each of these pipe sections had four windows (5 cm high x 6 cm wide) cut into the sides, which was covered by 1 µm nylon mesh (Normesh Ltd., Oldham, UK). The windows were positioned such that after insertion to the soil, they were just below the soil surface, and extending throughout the organic horizon into the mineral soil. The same design of pipe sections with windows was used for the *SM* treatment, but mesh size was increased to 41 µm. This aperture size allows fungal mycelium to penetrate into the soil enclosed within pipe sections from surrounding soil, but prevents ingress of roots (Heinemeyer et al., 2012). For the *SMR* treatment (i.e. intact rhizosphere control), we used shorter pipe sections (20 cm diameter, 8 cm length) inserted into the organic soil layer to about 2 cm depth. The emplacement of the PVC pipe sections for all treatments resulted in about 5-6 cm of pipe length extending above the soil surface (from here referred to as 'collars'), from where gas exchange with the atmosphere could be measured.

Collar locations were randomized within an area of approximately 300 m<sup>2</sup> within the forest stand, with a requirement of individual locations being between 50 and 200 cm from tree stems, and a minimum distance of 100 cm between collars. The different rhizosphere treatments were randomly allocated according to a block design (based on soil CO<sub>2</sub> efflux measurements from the soil surface prior to treatment allocation) in order to account for localized environmental effects. All collars were established 12 months prior to the flux

measurements to allow for a re-establishment of soil microbial communities following disturbance from collar installations, including the establishment of new ECM hyphal ingrowth in the *SM* treatment.

Both the amount of litter and the amount of precipitation entering collars was standardised to remove the influence of the considerable spatial heterogeneity on litter amounts and canopy through-fall. Collars were sheltered from through-fall using transparent shields of corrugated PVC (30 x 40 cm) suspended at about 25 cm above collars, and average amounts of rainfall (based on measurements on site) were added to collars every week.

#### *Soil CO<sub>2</sub> and CH<sub>4</sub> flux measurements*

From 5<sup>th</sup> May until 13<sup>th</sup> June 2009, soil surface fluxes of CO<sub>2</sub> and CH<sub>4</sub> were measured using 12 opaque multiplexed automatic chambers (LI-8100-101, Li-Cor, Lincoln, Nebraska, USA; approximately 20 cm diameter). Chambers were placed over PVC collars of respective treatments, sealing tightly around the outside of collars with a rubber gasket. CO<sub>2</sub> concentrations were measured using a LI-8100 (Li-Cor, Lincoln, Nebraska, USA), whilst CH<sub>4</sub> concentrations were measured using a Fast Greenhouse Gas analyser (FGGA; Los Gatos Research, Mount View, California, USA). The multiplexer sampled each chamber sequentially such that chambers were measured once an hour. During the measurements, each chamber was closed for 3 minutes only, ensuring that the enclosed soil area is subject to the same conditions as the surrounding soil.

#### *Environmental measurements*

Soil temperature and soil water content (SWC) were recorded every 10 minutes using PT100 thermistor probes and SM200 probes (Delta-T Devices, Cambridge, UK), respectively. Soil temperature measurements were at 0.05 and 0.1 m depths (n = 3 per depth) and SWC measurements (n = 3) were measured at 0.05 m depth m. Atmospheric pressure was recorded continuously (1 Hz) by the (LI-8100). Photosynthetically Active Radiation (PAR) was



measured every 10 minutes at a nearby canopy opening (QS5 PAR Quantum Sensor, Delta-T Devices, Cambridge, UK).

Additionally, SWC was measured inside all soil collars once a week prior to manual water addition (see above) using a hand-held probe (SM200, Delta-T Devices, Cambridge, UK). A spatial average of throughfall at the site were collected from the nine collectors (funnel diameter = 20 cm) once every week. These funnels were placed on the ground at random locations throughout the site.

Data for wind speed and wind gust speed were obtained from the UK Met-Office website ([www.metoffice.gov.uk](http://www.metoffice.gov.uk)) for observations from Linton on Ouse, located approximately 20 km NW of the experimental plot. Note that despite the spatial separation, these data are used to allow a general characterisation of atmospheric mixing due to wind, not precise conditions at the site (see below).

#### *Data processing and flux calculations*

Fluxes of CO<sub>2</sub> and CH<sub>4</sub> were calculated from linear regression of the concentration measurements obtained during each 3 minute chamber closure. The first 40 seconds of each measurement were removed to allow the complete mixing of chamber air, meaning that each regression used 140 data points spanning a 140 second period. The correlation coefficient ( $r^2$ ), root mean square error ( $RSME$ ) and  $p$  value were calculated for each linear regression.

In order to separate valid flux measurements from possible artefacts (e.g. due to incomplete chamber closure, or leakage), we removed all CO<sub>2</sub> and CH<sub>4</sub> flux estimates where the  $r^2$  value of the CO<sub>2</sub> measurement was below 0.9. This procedure removed approximately 19% of all data, most of which were associated with malfunctioning chambers during some of the observation period. Owing to the relatively smaller signal-to-noise ratio, small flux rates tended to show lower coefficients of variation ( $r^2$ ). This was more pronounced for methane flux calculations, due to the smaller absolute concentration changes for this flux, and we did not apply the same rigorous  $r^2$  threshold to fluxes as we did for CO<sub>2</sub>. Instead, any CH<sub>4</sub> flux with an  $RSME$  of more than 0.02  $\mu\text{mol m}^{-2} \text{s}^{-1}$  was also removed, affecting a further 1.8% of

flux values. Concentrations of CO<sub>2</sub> and CH<sub>4</sub> c. 0.1 m above the soil surface were recorded from each chamber location immediately before chamber closure (initial 5 readings for each channel, i.e. before the concentrations had increased).

Small gaps in the data series of each chamber (less than six consecutive hours) were filled by using the average of fluxes four hours before and after the gap (from the same chamber). Larger data gaps were not filled. Flux values were calculated for each chamber separately and averaged according to treatment (*S*, *SM*, *SMR*), using each chamber as a true replicate.

#### *Statistical methods*

Cumulative flux sums were analysed by means of a two-way Analysis of Variance (ANOVA) for each chamber to look for a block and treatment effect, and a post-hoc Duncan's MRT test applied, if the data met the assumptions of homogeneity of variance and normality. All flux calculations and statistical analysis of cumulative flux values was carried out using SAS v8.01 (Statistical Analysis Software). Correlations between concentrations, fluxes and environmental variables were carried out using the Spearman's rank method (owing to non-normal distributions) in the SPSS Statistics software (Version 21; IBM Corp.).

The relationships between continuous environmental variables and trace gas fluxes were investigated using linear and/or multiple regressions and analysis of covariance. In some cases, autoregressive (AR) models were employed because gas fluxes and environmental variables showed temporal autocorrelations. Residuals from exploratory regression modelling revealed strong autocorrelation for all fluxes, as confirmed by autocorrelation function (ACF) plots and the Durban Watson test (in all cases p-value < 0.001). It was found that a 2<sup>nd</sup> order AR model was optimal based on inspection of ACF plots. To facilitate comparisons between fitted coefficients, all variables were normalised by scaled to a mean of zero and a standard deviation of 1. The independent variables included in the regression models were: initial concentration of CO<sub>2</sub> or CH<sub>4</sub> (respectively), air pressure, air temperature, soil temperature (at 5 cm depth), solar radiation and soil water content.

## 229 Results

### 230 *Soil respiration*

231 Mean soil CO<sub>2</sub> flux (*SMR*) over the measuring period was  $0.91 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$ . For the  
232 rhizosphere treatments, we found a significant effect of treatment but no effect of block  
233 ( $F_{2,10} = 13.41$ ,  $P < 0.002$ ). Treatment *SMR* showed significantly higher CO<sub>2</sub> fluxes than either  
234 of the other two treatments (Table 1).

235 The overall heterotrophic contribution to soil respiration averaged  $55.2 \pm 0.3\%$  over the  
236 entire measurement period, with a tendency towards higher relative heterotrophic  
237 contributions towards the end of the observation period (Fig. 1c). Of the autotrophic  
238 contributions, about one-third could be attributed to ECM-mycelium CO<sub>2</sub> flux, with the  
239 remainder originating from roots ( $15.8 \pm 0.3\%$  and  $29.0 \pm 0.4\%$  of total soil CO<sub>2</sub> flux,  
240 respectively). Note that this is a simplistic presentation of flux contribution, based on flux  
241 differences to illustrate relative flux magnitudes. It assumes that flux contributions are  
242 independent and hence additive, thus excluding possible interactions between autotrophic  
243 and heterotrophic dynamics in the soil environment.

244 Over the course of the sampling period, soil CO<sub>2</sub> fluxes showed a gradual increase  
245 corresponding with seasonal changes in air and soil temperatures (Fig. 1d). At diurnal  
246 timescales, however, soil CO<sub>2</sub> flux showed lower rates at around midday, with flux rates  
247 reaching a peak at about 20:00 on average for the entire measurement period (Fig. 2b). The  
248 different rhizosphere treatments also show different diurnal patterns. For example, *SMR* and  
249 *SM* treatments show a more pronounced reduction in CO<sub>2</sub> flux during the middle of the day  
250 compared to the *S* treatment, resulting in greater diurnal amplitudes both in absolute and  
251 relative terms.

252

### 253 *Soil CH<sub>4</sub> uptake*

254 Mean CH<sub>4</sub> flux (*SMR*) over the measuring period was  $-1.63 \pm 0.22 \text{ nmol m}^{-2} \text{s}^{-1}$ . Soil CH<sub>4</sub> flux  
255 varied significantly among rhizosphere treatments, but no significant effect of block was

found (ANOVA  $F_{2,10} = 14.39$ ,  $P < 0.002$ ). The strongest sink was observed for the *SMR* treatment, followed by *SM* and *S* treatments ( $P < 0.01$ ; Table 1).

Unlike  $\text{CO}_2$  efflux,  $\text{CH}_4$  uptake did not show a gradual seasonal increase with rising temperatures. Instead, the  $\text{CH}_4$  sink strength showed short-term decreases following rain events and a gradual increase following the onset of drier conditions (Fig. 1e). On diurnal timescales, we observed a marked pattern of higher night-time  $\text{CH}_4$  oxidation rates and lower daytime fluxes (Fig. 2a). In contrast to  $\text{CO}_2$  dynamics, the daily oscillation in  $\text{CH}_4$  fluxes did not vary among rhizosphere treatments. Atmospheric  $\text{CH}_4$  concentrations measured above the soil surface showed lower daytime concentrations and higher night-time concentrations.

Spearman's rank correlation analysis indicated that there was a significant correlation between the rate of  $\text{CH}_4$  uptake in the soil and  $\text{CH}_4$  concentrations measured in the atmosphere above the soil surface (Fig. 3a). This correlation was significant for the entire data set ( $r = -0.237$ ;  $p < 0.01$ ;  $n = 759$ ), but was dominated by a strong dependence of fluxes on concentration at low soil water content ( $\text{SWC} = 0.22 - 0.35 \text{ m}^3 \text{ m}^{-3}$ ;  $r = -0.493$ ;  $p < 0.001$ ;  $n = 262$ ). Variation in  $\text{CH}_4$  concentration in the atmosphere above the soil surface was found to correlate in turn with wind speed (Fig. 3b).

#### *Relationship between trace gas fluxes and environmental variables*

The AR model indicates a significant effect of SWC dynamics on fluxes of  $\text{CO}_2$  and  $\text{CH}_4$  for all treatments (Table 2). For  $\text{CO}_2$ , fluxes increased with rising soil moisture, while the opposite pattern was true for  $\text{CH}_4$  (i.e. reduced  $\text{CH}_4$  uptake with increasing SWC). AR analysis also indicated that soil temperature at the 5 cm depth was a good predictor of soil  $\text{CO}_2$  fluxes among all the rhizosphere treatments, while air temperature was found to be a good predictor of  $\text{CH}_4$  fluxes (Table 2). Furthermore, a significant negative correlation was found between solar radiation and  $\text{CO}_2$  fluxes (Table 2).

### *Sampling frequency analysis*

Re-sampling the data set to simulate results that would have been obtained under contrasting sampling scenarios show a generally lower apparent CH<sub>4</sub> oxidation flux rate, with an apparent reduction by up to 14.5% for fortnightly sampling frequencies from the *SMR* treatment, 12.5% for alternate days in the *S* treatment, and 23.2% for fortnightly sampling from the *SM* treatment (Fig. 4). The CO<sub>2</sub> reduction in apparent flux was up to 13.8%, 17.9% and 12% for weekly sampling of *SMR*, *SM* and *S* treatments, respectively. The standard deviation associated with different sampling frequencies increases with decreasing frequency, owing to the lower number of sampling events for lower frequencies. Sampling frequencies of 1 and 2 weeks would have resulted in an under-estimation of mean CH<sub>4</sub> oxidation of 12.7 and 14.5%, respectively, compared to the 1-hour results in the *SMR* treatment. The uncertainty of estimates measured by the observed standard deviation of measurements for contrasting sampling intensities was similar for frequencies down to bi-weekly samplings. For less frequent intervals, standard deviations increased by approximately 25 and 50% for 1 and 2-week intervals, respectively.

## **Discussion**

### *Rhizosphere effects on soil CO<sub>2</sub>*

Results from the root and extraradical ECM mycelium exclusion treatments suggest a significant effect of root and ECM presence on CO<sub>2</sub> flux. Higher soil CO<sub>2</sub> efflux in the *SMR* treatment can be expected, and has been documented exhaustively elsewhere in other soil respiration partitioning studies (Subke et al., 2006). The enhanced soil CO<sub>2</sub> flux in the *SMR* treatment reflects the respiration of live roots and mineralization of root-derived organic materials in the rhizosphere, and the proportion of heterotrophic respiration ( $51.1 \pm 13.6\%$ ) falls within the range observed in other temperate forest sites (Subke et al., 2006; Bond-Lamberty and Thomson, 2010). The lack of a significant difference between *SM* and *S* treatments, while surprising, may reflect the fact that the mycorrhizal biomass in *SM* treatments was not large enough to produce significantly greater amounts of CO<sub>2</sub> compared to the *S* treatment. The mesh-collar approach we chose for this study selects in-growth

based on hyphal diameter only, but we acknowledge that it creates further selection of ECM species based on their “exploration types” (Tedersoo and Smith, 2013); whilst species classified as long to medium distance explorers (*sensu* Tedersoo & Smith, 2013) are likely to dominate in *SM* treatments, ‘contact’ and short-distance explorer types of ECM are likely to be underrepresented.

#### *Rhizosphere effects on soil CH<sub>4</sub>*

What was more intriguing, however, was the distinct pattern in CH<sub>4</sub> uptake among the root & ECM exclusion treatments. In the presence of a fully intact rhizosphere (*SMR* treatment), net CH<sub>4</sub> uptake was almost 3 times that of the bulk soil; while in the presence of ECM hyphae, net CH<sub>4</sub> uptake was approximately 40% higher than in the bulk soil (Table 1). Although some of this variation in fluxes may be attributable to differences in soil moisture content among the treatments (see section on the role of environmental drivers below), we believe it is unlikely that soil moisture was the principal cause for this pattern because the absolute difference in soil moisture content among the treatments was small compared to the difference in fluxes (e.g. soil moisture varied by only 1.5-13.0 %, whereas CH<sub>4</sub> fluxes varied by as much as 300 % among treatments). Other measured environmental variables did not vary significantly between treatments. This suggests that the observed pattern was due to some other biotic or environmental factor that we did not measure, or the result of fundamental underlying differences in microbial methanotrophic populations among treatments. With respect to the latter, we propose that soil with an intact rhizosphere may promote a more vigorous methanotrophic community by supplying methanotrophs with alternate sources of labile C (e.g. methanol, formaldehyde, formate) and/or by providing a greater sources of nutrients for methanotroph growth (Hanson and Hanson, 1996; Bodelier and Laanbroek, 2004; Veraart et al., 2015). Highest fine root densities in this forest occur throughout the organic horizon and superficial mineral horizons; soil methanotrophic bacteria are generally assumed to occur mainly in the upper mineral horizons in coniferous forests (Saari et al., 1998), so the close spatial proximity makes it possible that rhizosphere derived C<sub>1</sub> compounds support the population size of also methanotrophs. In addition, roots and extraradical ECM hyphae can also promote macropore and aggregate formation

(Angers and Caron, 1998; Six et al., 2006), which may facilitate transport of CH<sub>4</sub> to methanotrophs by improving soil structure and overall pore connectivity.

#### *Environmental regulation of CO<sub>2</sub> flux*

Mean CO<sub>2</sub> flux ( $0.91 \pm 0.07 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) is close to the mean of boreal forests ( $1.01 \pm 0.60 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), but in the lower range of annual temperate forest rates ( $1.97 \pm 1.11 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Bond-Lamberty and Thomson, 2010). Both soil temperature and soil water content (with the exception of the *Soil* treatment) significantly influenced the dynamics of soil CO<sub>2</sub> efflux, consistent with studies in other forest ecosystems (Wu et al., 2011). However, what was surprising is an apparent negative correlation between radiation and soil CO<sub>2</sub> efflux (Table 2). The temporal shift in peak soil CO<sub>2</sub> efflux, which occurs between 18:00 and 20:00 h, may in part explain this correlation, as periods of high radiation are associated with low CO<sub>2</sub> flux, and peak fluxes occurred close to the time of sun set. However, the autoregressive model showed a strong influence of soil temperature, which also peaked between 18:00 and 20:00, so that the additional influence of radiation remains unexplained. We note that the *S* treatment (which does not experience direct influence of belowground allocation of C by plants) does not show any statistically significant effect of radiation, which suggests that the inverse radiation-CO<sub>2</sub> flux relationship is influenced by autotrophic C supply. Why this should have a negative sign is however less clear, as previous studies have established a clear and direct relationship between radiation (and hence photosynthetic activity) and belowground CO<sub>2</sub> fluxes (Mencuccini and Hölttä, 2010; Martin et al., 2012). One possible explanation is that night-time depletion of sugars or other carbohydrate stores may suppress carbohydrate utilisation (and consequently, respiration) during the first half of the day, leading to the apparent negative relationship between radiation and root respiration earlier in the day (Gibon et al., 2004). Subsequent accumulation of photosynthate may release this biochemical inhibition, leading to higher respiration rates during the evening and night.

Lags between C assimilation in the canopy and utilisation in the rhizosphere are a further possibility to explain shifts in fluxes with regards to drivers. The meta-analysis of transport times of sugars fixed during photosynthesis to root via the phloem by Mencuccini and Hölttä

(2010) indicates that for an approximate 10 m path length (tree height plus root length), a lag of between 1 and 3 days is likely. However, the observation that peak CO<sub>2</sub> flux in the S treatment coincides with that in other (autotroph-influenced) treatments (Fig. 2b) suggests that, whilst the magnitude of response is impacted by photosynthate supply, the timing is more likely to relate to lags in soil diffusion.

#### *Environmental regulation of CH<sub>4</sub> flux*

The magnitude of CH<sub>4</sub> uptake in intact soil collars over the sampling period ( $1.63 \pm 0.22 \text{ nmol m}^{-2} \text{ s}^{-1}$ , Table 1) is similar to fluxes reported from mixed deciduous woodlands in Scotland (0.14 to  $2.39 \text{ nmol m}^{-2} \text{ s}^{-1}$  (Dobbie et al., 1996), but relatively high when compared to fluxes across other European temperate forests (uptake rates of 0.18 to  $1.43 \text{ nmol m}^{-2} \text{ s}^{-1}$  averaged over an entire year; (Grunwald et al., 2012). Our results indicate a significant influence of soil moisture and air temperature on CH<sub>4</sub> flux over the measurement period, confirming findings from another temperate coniferous site (Ueyama et al., 2015). Unlike CO<sub>2</sub>, the rate of CH<sub>4</sub> uptake was inversely related to both soil moisture and air temperature; i.e. the positive correlation between CH<sub>4</sub> flux and soil moisture or air temperature represents an inverse relationship with CH<sub>4</sub> uptake because more negative fluxes denote higher rates of CH<sub>4</sub> oxidation while more positive fluxes denote lower rates of CH<sub>4</sub> oxidation. For example, over the moisture range observed in this experiment, CH<sub>4</sub> uptake declined in response to rising soil moisture content (i.e. CH<sub>4</sub> flux became more positive with increasing soil moisture). Progressive drying of soil probably increased soil pore connectivity and facilitated more rapid transport of CH<sub>4</sub> from the atmosphere to sites of methanotrophic activity (see late May, early June in Fig. 1). Likewise, increases in air temperature were associated with a decline in rates of CH<sub>4</sub> uptake (i.e. CH<sub>4</sub> flux also became more positive with increasing air temperature). This trend may reflect the effect of temperature on CH<sub>4</sub> dissolution and substrate supply to methanotrophs. Methane is a poorly soluble hydrophobic compound, and its dissolution into the aqueous phase is closely linked to temperature. Higher air temperatures may reduce rates of CH<sub>4</sub> dissolution, subsequently reducing the supply of aqueous-phase CH<sub>4</sub> to methanotrophs and thus suppressing rates of atmospheric CH<sub>4</sub> uptake (Teh et al., 2006; Templeton et al., 2006).



Alternatively, the apparent inverse relationship between air temperature and CH<sub>4</sub> flux may be a result of the concurrent diurnal fluctuations in atmospheric CH<sub>4</sub> concentrations (Fig. 2), which may obscure a confounding impact of substrate limitations underlying the CH<sub>4</sub> flux response (see below).

The observed influence of soil moisture on CH<sub>4</sub> uptake slightly complicates a direct interpretation of rhizosphere treatments. Manual soil moisture measurements showed a significant (although numerically small) influence of treatment on soil moisture, with the *SMR* treatment having consistently lower soil moisture than the other two treatments. This artefact from deep collar methods has been reported before (Heinemeyer et al., 2012), and is likely to be caused by the absence of root water uptake in *SM* and *S* treatments. However, the magnitude of the treatment effect on soil moisture, whilst statistically significant, is small (between 0.01 and 0.03 m<sup>3</sup> m<sup>-3</sup> for a soil water content range of between 0.23 and 0.66 m<sup>3</sup> m<sup>-3</sup> over the measuring period). The relatively consistent contributions of autotrophic sources to soil CO<sub>2</sub> efflux (Fig. 1c) suggest that the soil moisture variations were insufficient to impact on plant productivity and rhizosphere C allocation, so that microbial supply of plant-derived C did not seemingly change significantly over the measurement period, notwithstanding an apparent reduction in root and ECM flux contributions in the last week in Fig. 1c.

Interestingly, there was also a significant and well-constrained influence of CH<sub>4</sub> concentration on CH<sub>4</sub> uptake, with CH<sub>4</sub> uptake increasing (i.e. fluxes becoming more negative) with increasing CH<sub>4</sub> concentration. Diurnal changes in CH<sub>4</sub> concentration were therefore associated with predictable diurnal shift in CH<sub>4</sub> uptake. For example, daytime mean concentrations of CH<sub>4</sub> were consistently around 1.86 ppm between the hours of 9:00 and 20:00, but night-time concentrations showed progressively increasing concentrations, with a peak of c. 1.95 ppm at 6:00. This diurnal variation in CH<sub>4</sub> concentrations coincides with an overall shift towards higher CH<sub>4</sub> uptake rates at night. The underlying cause for this shift towards higher nighttime CH<sub>4</sub> concentrations are atmospheric mixing effects. Collapse of the atmospheric boundary layer at night and poorer atmospheric mixing leads to the localized accumulation of atmospheric CH<sub>4</sub> (Baldocchi et al. 2012). However, given the consistent and comparatively strong soil CH<sub>4</sub> sink, the nighttime increase in local atmospheric CH<sub>4</sub> concentrations above the global tropospheric average is surprising. We can

only speculate that the increase in concentration could be caused by local hotspots of CH<sub>4</sub> production located away from the immediate measurement plot (Baldocchi et al., 2012). For example, CH<sub>4</sub> production from local anaerobic hotspots (Baldocchi et al., 2012) or soil-derived CH<sub>4</sub> emissions transported through trees (Covey et al., 2012; Wang et al., 2016) may enhance local atmospheric CH<sub>4</sub> concentrations under stable nighttime atmospheric conditions. Irrespective of the actual source of CH<sub>4</sub> underlying the increase during periods of low atmospheric mixing, there is a clear response in the strength of CH<sub>4</sub> uptake and atmospheric concentration, in good agreement in diurnal patterns (Fig. 2a & 2c). This finding is potentially significant, as it suggests that soil microbial oxidizers may represent a potential negative feedback to rising atmospheric CH<sub>4</sub> concentrations. Our observations are supported by a number of laboratory-based studies that have found clear methane oxidation dependencies when large concentration gradients are applied (Bender and Conrad, 1992; Tate et al., 2012; Malghani et al., 2016). Experimental ranges in these studies exceed concentration ranges normally encountered in the boundary layer above the soil surface; concentration ranges in cited publications are 40 – 570 ppm in Tate et al (2012), 30–60 ppm in Malghani et al (2016) or even 5% in Bender & Conrad (1992). That methane oxidation rates respond to much smaller variations in concentration detectable in the field is however a novel observation. Of course, one important caveat is that the AR model did not identify CH<sub>4</sub> concentration as a significant predictor of CH<sub>4</sub> flux, despite the strong correlation. As mentioned before, there is a possibility that confounding covariance of air temperature and CH<sub>4</sub> concentrations may obscure actual relationships between CH<sub>4</sub> flux and driving variables, and field-based experimental manipulations of methane concentrations and temperature are needed to resolve this point.

#### *Insights obtained from quasi-continuous chamber measurements*

Quasi-continuous, automated sampling of soil gas exchange provides the most comprehensive data to estimate soil or ecosystem greenhouse gas budgets. The sampling frequency exercise we performed indicated that manual chamber sampling, assuming that manually sampled fluxes were collected during mid-day, under-estimate soil CO<sub>2</sub> and CH<sub>4</sub> fluxes from our temperate forest study site by 12–15 %. This is because manual sampling

during day-time hours would not have accounted for diurnal changes in gas flux, in particular periods when gas fluxes were heightened (e.g. enhanced soil respiration between 18:00-20:00 and elevated CH<sub>4</sub> uptake from 20:00-6:00). Continuous atmospheric flux measurements (such as the eddy covariance technique) provide a further powerful tool to investigate short-term temporal flux variations and dependence on environmental drivers(Phillips et al., 2017), but chamber based studies like ours provide critical process understanding from manipulations that can not be captured by eddy covariance.

It should be noted that these are not universal values that can be applied to correct manual gas sampling estimates obtained in other temperate forest locations. Rather, it serves to illustrate that diurnal fluctuations in soil gas exchange should be obtained for studies otherwise relying on periodic gas sampling in order to estimate seasonal or annual budgets in order to account for fluxes that may be partially driven by recurring (e.g. diurnal) shifts in environmental conditions or circadian patterns.

A key insight gained from the use of this continuous sampling approach is that we have identified temporal trends in the data that may point to new or previously unidentified controls on CH<sub>4</sub> and CO<sub>2</sub> fluxes. The mid-day depression in soil respiration and the subsequent rise in fluxes from 18:00-20:00 may suggest a physiological control on autotrophic respiration linked to the internal carbohydrate status of plant tissues (Gibon et al., 2004), whilst the night-time increase in soil CH<sub>4</sub> uptake, coincident with the rise in atmospheric CH<sub>4</sub> concentrations, may indicate that high-affinity CH<sub>4</sub> oxidising bacteria are sensitive to small and short-term variations in substrate availability, a phenomenon not described before.

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638 **Table 1** Mean flux rates of methane and CO<sub>2</sub> for collars with contrasting access by roots  
639 and/or mycorrhizal hyphae: *SMR* = “soil, extraradical ECM hyphae & roots”, *SM* = “soil 7  
640 extraradical ECM hyphae”, and *S* = heterotrophic soil CO<sub>2</sub> flux. Values are averages (± 1 St.  
641 Error) using temporal averages of flux rates from n = 4 collars as replicates. Different lower-  
642 case letters indicate significant differences between treatments for each of the gases.

Treatment	Mean CO <sub>2</sub> flux (μmol m <sup>-2</sup> s <sup>-1</sup> )	Mean CH <sub>4</sub> flux (nmol m <sup>-2</sup> s <sup>-1</sup> )
<i>SMR</i>	0.9061 ± 0.0705 <sup>a</sup>	-1.626 ± 0.221 <sup>a</sup>
<i>SM</i>	0.6521 ± 0.0317 <sup>b</sup>	-0.8180 ± 0.1216 <sup>b</sup>
<i>S</i>	0.5352 ± 0.0454 <sup>b</sup>	-0.5877 ± 0.0530 <sup>c</sup>

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1 **Table 2** Coefficients from the autoregressive (AR) model. Coefficients of each parameter are shown along with the standard error (S.E.).  
2 Significance Coefficients are highlighted in bold with the level of significance indicated: p <0.001 (\*\*\*), p <0.01 (\*\*), p <0.05 (\*) and for  
3 marginally insignificant Coefficients p <0.1 (#). Note that all variables were scaled to a mean of zero and a standard deviation of 1.

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		Intercept	AR(1)	AR(2)	Initial CO <sub>2</sub>	Initial CH <sub>4</sub>	SWC	Pressure	Radiation	T <sub>air</sub>	T <sub>soil</sub>	Adj-R <sup>2</sup>
F <sub>CO2_S</sub>	Coeff	0.021	<b>0.629***</b>	<b>0.298***</b>	-0.026		0.031#	-0.017	-0.028	-0.004	<b>0.055**</b>	<b>0.88***</b>
	S.E.	0.014	<b>0.038</b>	<b>0.038</b>	0.017		0.016	0.020	0.018	0.024	<b>0.021</b>	
F <sub>CO2_MS</sub>	Coeff	<b>0.041*</b>	<b>0.596***</b>	<b>0.220***</b>	-0.001		<b>0.080***</b>	-0.002	<b>-0.063**</b>	0.001	<b>0.095***</b>	<b>0.80***</b>
	S.E.	<b>0.018</b>	<b>0.040</b>	<b>0.040</b>	0.022		<b>0.022</b>	0.025	<b>0.022</b>	0.030	<b>0.027</b>	
F <sub>CO2_RMS</sub>	Coeff	<b>0.049**</b>	<b>0.610***</b>	<b>0.171***</b>	0.031		<b>0.079***</b>	-0.035#	<b>-0.077***</b>	0.009	<b>0.139***</b>	<b>0.86***</b>
	S.E.	<b>0.015</b>	<b>0.039</b>	<b>0.038</b>	0.019		<b>0.018</b>	0.020	<b>0.018</b>	0.023	<b>0.023</b>	
F <sub>CH4_S</sub>	Coeff	0.013	<b>0.589***</b>	<b>0.269*</b>	-	0.007	<b>0.071**</b>	0.035	-0.032	<b>0.083*</b>	-0.045	<b>0.74***</b>
	S.E.	0.022	<b>0.038</b>	<b>0.038</b>		0.023	<b>0.027</b>	0.031	0.027	<b>0.034</b>	0.028	
F <sub>CH4_MS</sub>	Coeff	0.008	<b>0.524***</b>	<b>0.330***</b>	-	0.002	0.049#	0.027	<b>-0.056*</b>	<b>0.107**</b>	-0.043	<b>0.72***</b>
	S.E.	0.022	<b>0.038</b>	<b>0.037</b>		0.023	0.026	0.032	<b>0.027</b>	<b>0.034</b>	0.029	
F <sub>CH4_RMS</sub>	Coeff	-0.002	<b>0.610***</b>	<b>0.308***</b>	-	0.015	<b>0.052**</b>	0.014	0.021	<b>0.057*</b>	-0.046*	<b>0.88**</b>
	S.E.	0.015	<b>0.037</b>	<b>0.036</b>		0.015	<b>0.019</b>	0.021	0.018	<b>0.023</b>	0.019	

5

## 1 **Figure captions**

### 2 *Figure 1*

3 Overview of flux dynamics and environmental parameters during the measuring  
4 period. (a) CH<sub>4</sub> flux and (b) CO<sub>2</sub> flux from *SMR* (black), *SM* (grey) and *S* (open) collars.  
5 (c) Apparent CO<sub>2</sub> flux fractions from decomposition (light grey), extraradical ECM  
6 hyphae (dark grey) and “true” root respiration (black), based on flux difference  
7 between collar treatments. (d) Temperatures measured in the soil at 5 cm (grey line)  
8 and 10 cm (black line) and in the air above the soil surface (dashed line). Soil water  
9 content was measured continuously (n = 3) (e), and periodically for different  
10 treatments (f). All error bars represent 1 standard error (n = 4).

### 11 *Figure 2*

12 Mean diurnal dynamics of CH<sub>4</sub> and CO<sub>2</sub> fluxes and key environmental parameters.  
13 Data are means averaged over the entire measuring period, thin lines indicate the  
14 95% confidence intervals; maximum and minimum values are indicated by grey and  
15 white circles, respectively (meaning min. and max. *negative* fluxes for CH<sub>4</sub>). Mean  
16 hourly fluxes of CH<sub>4</sub> (a) and CO<sub>2</sub> (b) for the three collar types are shown alongside  
17 CH<sub>4</sub> and CO<sub>2</sub> concentrations above the soil surface (c, d). Collar treatments are  
18 shown separately in panels a and b: *SMR* (solid black lines), *SM* (grey lines), and *S*  
19 (dashed black lines). Also shown are diurnal courses of air temperature (e), and soil  
20 temperature at 5 cm depth (f).

### 21 *Figure 3*

22 (a) Relationship between CH<sub>4</sub> concentration above the soil surface and wind speed.  
23 (b) Correlation between CH<sub>4</sub> concentration above the soil surface and instantaneous  
24 CH<sub>4</sub> flux in *SMR* treatment. The main graph shows correlation of driest conditions  
25 (Soil Water Content between 0.22 and 0.35 m<sup>3</sup> m<sup>-3</sup>), inset shows all data.

### 26 *Figure 4*

27 Mean CH<sub>4</sub> (a) and CO<sub>2</sub> (b) flux estimate for all three treatments over the 6-week  
28 observation period based on increasing sampling intervals. Horizontal lines give the  
29 “true” average flux based on hourly observations. Black symbols & solid lines: *SMR*,  
30 grey symbols and lines: *SM*, open symbols and hatched lines: *S*; error bars show

31 standard errors. Numbers of temporal replicates for each sampling interval (identical  
32 for all collar treatments and both gases) is indicated in the upper panel.

33